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USE OF EXOGENOUS PURINES AND PURINE NUCLEOTIDES IN THE BIOSYNTHESIS OF NUCLEIC ACIDS IN PLAGUE BACTERIA

Various species of live cells differ significantly in their capacity for assimilation and inter-conversion of exogenous purines and their derivatives /1,2/, therefore the obtaining of data concerning the peculiarities of these processes in new objects is of definite interest. The study of metabolism of purines in the plague microbe is also important because a number of investigators /3, 4/ connect the metabolism of nitrogenous bases in this microbe with its virulence.

The purpose of the present investigation was clearing up the capacity of the plague microbe for assimilation and inter-conversion of purines and purine nucleotides in the process of biosynthesis of nucleic acids.

Methods of Investigation

The object of investigation was the plague microbe, strain EV, line NIIEG.

Incubation was carried out on "whole" synthetic medium which was proposed by I. V. Domaradskiy and V. A. Ivanov /5/, however the concentration of amino acids was reduced by 10 times, which, as special tests showed, did not have a significant influence on the incorporation of label and the accumulation of nucleic acids in the first hours of growth.

Incubation was carried out at 28° under conditions of aeration with intensive shaking. Seeding dose was $1 \cdot 10^9$ microbial cells in 1 ml.

Incubation time was 4 hours. As it was established earlier /6/, by this time (end of the lag-phase of growth) the content of RNA in cells of the plague microbe was approximately doubled and the main portion of $HC^{14}OONa$ label incorporated in the bacteria is revealed in the nucleic acids.

The radioactive substances used were: $HC^{14}OONa$ with a specific activity of 43.8 and 48.3 $\mu Ci/g$, $8-C^{14}$ adenine 9.4 $\mu Ci/g$, $8-C^{14}$ guanine 1.1 $\mu Ci/g$, and $8-C^{14}$ xanthine 16 $\mu Ci/g$.

Radioactive substances and other additions were introduced into the incubation medium in the form of aqueous solutions in equimolecular amounts (usually in the limits of 20 $\mu g/ml$).

The remaining methods of investigation (obtaining of bacterial mass, isolation of RNA and DNA, separation of mononucleotides of RNA and nitrogenous bases of DNA, radiometry, etc.) were described earlier [6].

Relative specific activity (RSA) of purines of freshly synthesized RNA (RSA_f) was calculated by the formula:

$$RSA_f = \frac{c}{c - c_0} \cdot RSA,$$

where c_0 and c - content of RNA in cells of the plague microbe in the beginning and end of incubation respectively.

Results of the Investigation

Purines and purine nucleotides which were introduced into the incubation medium, with the exception of guanine, had no significant influence on the content of nucleic acids in cells of the plague microbe after 4 hours of cultivation.   The quantity of RNA, which comprised around 4% in the inoculum, approximately doubled over this time and comprised 6.4--8.3%, the quantity of DNA was relatively constant and comprised 2--2.5% to the weight of dry bacteria.   With the addition of guanine the content of nucleic acids was higher (RNA 9.4%, DNA 2.6%).

Regardless of the radioactive substance used the incorporation of label in nucleic acids in all cases comprised 55--80% of all the activity revealed in bacterial cells.   Of this around 10% was incorporated in DNA.

Table 1 presents data on the influence of purines and purine nucleotides on the incorporation of $HC^{14}OONa$ in bacterial cells and purines of nucleic acids of the plague microbe.   It was established earlier [6] on this same object that incorporation of $HC^{14}OONa$ characterizes the synthesis of purines from simple compounds.

It is apparent from Table 1 that incorporation of C^{14} in cells of bacteria which were incubated on a medium without purines and their derivatives is proportional to the specific activity of the medium.

2.

Table 1

Influence of exogenous purines and purine nucleotides on the incorporation of $HC^{14}OONa$ in bacterial cells and purines of nucleic acids of the plague microbe

(a) Добавка к среде	(b) УА среды (в мккюри/мл)	(c) УА бактерий (в имп/мин·мг·10⁻³)	(d) ОУА			
			(e) РНК		(f) ДНК	
			Аденин	Гуанин	Аденин	Гуанин
—	0,40	248.4	5,8	6,8	2,15	2,06
Аденин	0,40	156,1	—	9,4	—	1,54
Гуанин	0,40	167,1	8,5	2,6	2,3	0,77
Гипоксантин	0,40	42,2	1,47	1,27	0,56	0,51
Ксантин	0,40	150,1	5,1	3,4	1,75	1,17
АМФ	0,40	171,6	1,55	9,0	0,93	1,96
АДФ	0,40	200,9	5,0	6,1	1,4	1,23
АТФ	0,40	164,1	2,34	6,5	0,28	1,6
—	0,27	167,7	6,4	5,6	1,62	1,78
Гуанин	0,27	93,0	4,0	1,75	2,0	0,57
ГМФ	0,27	28,6	2,2	—	0,57	—
ГДФ	0,27	125,0	4,73	4,54	1,23	1,19
ГТФ	0,27	166,5	6,1	5,85	1,46	1,63
—	0,135	77,6	2,65	2,65	1,6	1,6
Гипоксантин	0,135	21,1	0,75	0,89	0,21	0,31
ИМФ	0,135	66,0	2,1	2,5	1,55	1,77
ИДФ	0,135	76,4	2,63	2,5	1,15	1,26
ИТФ	0,135	80,8	2,1	2,45	0,92	1,15

$$RSA = \frac{Imp/min \cdot \mu mole \text{ of NA purine}}{Imp/min \cdot 2 \mu mole \text{ of } HC^{14}OONa} \cdot 100.$$

In this case the calculation formula includes the coefficient 2, since in the biosynthesis of one purine ring 2 molecules of formate participate /1/.

Key: (a) Addition to the medium; (b) Specific activity of medium (in $\mu Ci/ml$); (c) Specific activity of bacteria (in imp/min · mg · 10^{-3}); (d) RSA; (e) RNA; (f) DNA; (g) adenine; (h) guanine; (i) hypoxanthine; (j) xanthine; (k) AMP; (l) ADP; (m) ATP; (n) GMP; (o) GDP; (p) GTP; (q) hypoxanthine; (r) IMP; (s) IDP; (t) ITP.

Exogenous purines (adenine, guanine, hypoxanthine, and xanthine) considerably lower the incorporation of $HC^{14}OONa$ in bacterial cells of plague microbe. It is known that the "screening" action of exogenous purines on the de novo synthesis from labeled precursors can serve as a sufficiently reliable method for measurement of their utilization /1/.

As it follows from Table 1, the RSA values for purines of RNA do not exceed 9.4%. Since freshly synthesized RNA in our tests comprised approximately half of all the RNA, then the quantity of purines, synthesized de novo with the participation of $HC^{14}OONa$, in freshly formed RNA comprised no more than 20%. The remaining share of polynucleotide purines is apparently formed from the intracellular background of NA precursors.

Exogenous adenine completely suppresses the synthesis of adenine from simple compounds, however the new growth of guanine continues here even more intensively. The addition of guanine suppresses the de novo synthesis of purine by more than 2 times and the synthesis of adenine from simple compounds is increased. These facts make it possible to assume that in the microbe under study a significant inter-conversion of adenine and guanine does not take place.

Exogenous hypoxanthine lowers the synthesis of both purines from simple compounds by more than 4-5 times. Apparently hypoxanthine can be converted by the plague microbe into both purines of nucleic acids at the same time that xanthine which is added is used primarily in the synthesis of guanine.

The action of AMP and ATP on the distribution of label between the purines of nucleic acids is analogous to the action of adenine, but less effective. Exogenous AMP suppresses more considerably the incorporation of adenine which is synthesized from simple compounds in RNA adenine, and exogenous ATP - in DNA adenine.

Of the remaining purine nucleotides only GMP significantly influences the incorporation and distribution of C^{14}-formate. The data from Table 1 permit the assumption that exogenous GMP is used not only in the synthesis of guanine, but also of adenine.

For confirmation of the positions stated above experiments were carried out with radioactive purine bases.

With the addition of $8-C^{14}$-adenine into the incubation medium all the radioactivity, incorporated in the polynucleotides of the plague microbe, is revealed only in the adenine of both nucleic acids. This fact is direct proof that the studied object cannot convert exogenous adenine into guanine of polynucleotides.

In Table 2 data are given concerning the influence of exogenous purines and adenine nucleotides on the incorporation of $8-C^{14}$-adenine in bacterial cells and adenine of nucleic acids of the plague microbe. From Table 2 it can be seen with dilution of labelled adenine by 2 times correspondingly there is a decrease in the specific activity of bacteria and adenine of nucleic acids. This fact indicates that in cells of the plague microbe there is an absence of or extremely small background of free adenine.

4.

Table 2

Influence of exogenous purines and purine nucleotides on the assimilation of 8-C^{14}-adenine by the plague microbe

(a) Добавка к среде	(b) УА бактерий (в ими/мин· ·мг·10⁻³)	(c) OVA аденина		
		(d) всей РНК	(e) вновь синтезированной РНК	(f) всей ДНК
—	109.5	38.6	80.3	5.2
(g) Аденин ..	53.6	20.2	49.3	3.0
(h) Гуанин ..	105.1	34.8	71.4	17.5
(i) Ксантин ..	101.4	35.5	67.6	6.2
(j) Гипоксантин	109.5	34.7	75.3	6.8
(k) АМФ ...	94.6	31.6	63.2	8.0
(l) АДФ ...	114.5	40.3	85.0	6.9
(m) АТФ ...	90.4	36.1	70.4	4.7

$$RSA = \frac{Imp/min \cdot \mu mole \text{ of nucleic acid adenine}}{Imp/min \cdot \mu mole \text{ of exogenous } C^{14}\text{-adenine}} \cdot 100 \cdot$$

Key: (a) Addition to medium; (b) Specific activity of bacteria (in imp/min · mg · 10⁻³); (c) RSA of adenine; (d) of entire RNA; (e) of synthesized RNA; (f) of entire DNA; (g) Adenine; (h) Guanine; (i) Xanthine; (j) Hypoxanthine; (k) AMP; (l) ADP; (m) ATP.

Exogenous guanine, while not exerting a significant influence on the incorporation of 8-C^{14}-adenine in bacteria and adenine of RNA, increases its incorporation in adenine of DNA. Of the remaining additives only AMP and ATP significantly lower the incorporation of C^{14}-adenine in cells of the plague microbe and adenine of RNA. Here, just as in the experiments with HC^{14}OONa, ATP and AMP influence the incorporation of adenine in RNA and DNA in a diverse manner.

Table 2 also cites the values of RSA of adenine of newly synthesized RNA. It follows from these data that exogenous adenine in our experiments is the main source of adenine of freshly synthesized RNA. Thus exogenous adenine, being incorporated easily in the adenine of nucleic acids, almost completely suppresses other routes of synthesis of adenine of polynucleotides, including the use of intracellular background of precursors.

In experiments with the addition of 8-C^{14}-guanine it was established that the latter is incorporated primarily in guanine of both nucleic acids. Thus the specific activity of DNA adenine is 10.6 times lower than the activity of DNA guanine. It follows from this that the EV strain of the plague microbe only to an insignificant degree transforms exogenous guanine to adenine, preferring to syn-

thesize adenine from simple compounds or using the intracellular background of its precursors.

Results of the investigation of bacterial cells, incubated in the presence of 8-C^{14}-xanthine, confirmed the assumption that xanthine is used primarily in the synthesis of guanine (Table 3).

Table 3

Influence of additives on the assimilation of 8-C^{14}-xanthine by the plague microbe

$$ RSA = \frac{\text{Imp/min} \cdot \mu\text{mole of purine of nucleic acids}}{\text{Imp/min} \cdot \mu\text{mole of exogenous C}^{14}\text{-xanthine}} \cdot 100 $$

Key: (a) Additive to medium; (b) Specific activity of bacteria (in imp/min · mg · 10^{-3}); (c) RSA; (d) RNA; (e) DNA; (f) adenine; (g) guanine; (h) Adenine; (i) Guanine; (j) Hypoxanthine; (k) GMP; (l) IMP.

As it follows from Table 3, in adenine only 10-15% radioactivity of purines of nucleic acids of the plague microbe is detected. The main portion of guanine of freshly synthesized nucleic acid may be formed from exogenous xanthine. Adenine, hypoxanthine, and GMP introduced into the medium of incubation completely suppress the incorporation of xanthine in adenine of polynucleotides. Exogenous guanine, hypoxanthine, AND GMP significantly inhibit the utilization of xanthine for the synthesis of guanine of polynucleotides of the plague microbe.

IMP did not significantly lower the incorporation both of HC^{14}OONa and xanthine in cells and nucleic acids of the plague microbe.

6.

Discussion of Results

In the event of incubation of the plague microbe in a medium which does not contain purine bases the synthesis of these substances from simple compounds takes place. This is testified to by the incorporation of $HC^{14}OONa$ in the composition of both purines of nucleic acids. However, by the end of the lag phase of growth the main source of purines is the intracellular background of their precursors.

According to the arrangement of B'yukenan [7], free purine bases are not intermediate products from the biosynthesis of purines of nucleic acids. Nevertheless in our experiments, just as in experiments with other objects, exogenous purines are incorporated in the purines of nucleotides easily, suppressing other paths of synthesis of one or both purines of nucleic acids.

Exogenous adenine in cells of the plague microbe suppresses the conversion of hypoxanthine into adenine (or IMP into AMP), but not the formation of the common precursor of purines of nucleic acids, since with the presence of adenine in the medium the formation of guanine from simple compounds and the intracellular background of its presursors continues. This example testifies to the fact that regulation of biosynthesis of purines in the plague microbe is realized by means of suppression of the reaction by its end product based on the principle of negative reverse bond.

In the object under study differences were clearly apparent in the utilization of exogenous purine bases. For the EV strain of the plague microbe the synthesis of purines of nucleic acids de novo turned out to be preferable, and not the interconversion of adenine and guanine. The lack of the ability on the part of the plague microbe for the conversion of adenine to guanine and the weak conversion by it of guanine to adenine are essential differences between the object studied and other microorganisms studied [1, 2, 8-10].

Other authors arrive at the conclusion concerning the absence in the plague microbe of the capacity to oxidize and deaminate purines [11]. At the same time plague microbe possesses the ability to deaminate a number pyrimidines and their derivatives [11, 12].

The absence of conversion of exogenous adenine to guanine, weak conversion of guanine to NA adenine, the absence or inertness of purine oxidase, etc. testify to the fact that the collection of enzymes taking part in the biosynthesis of nucleotides and nucleic acids in the object studied by us is more limited in comparison with a number of other microorganisms: E. coli, S. typhi, L. casei, etc.

Burrows [5] considers independence on supply of exogenous purines as one of the factors of plague microbe virulence.

The fact that the plague microbe prefers to utilize exogenous purines, and not the intracellular background of their precursors, testifies that exogenous purines may play an important role in the physiology of the plague microbe.

If the mechanism of assimilation of exogenous purines in cells of the plague microbe is analogous to the mechanism established for cell-less systems /2, 13, 14/, then in cells of the plague microbe there should be a high degree of activity of the corresponding pyrophosphorylases. However, it is not excluded that in an intact microbial cell other mechanisms of assimilation of exogenous purines may exist.

In studying the assimilation of purine nucleotides it is necessary to consider the presence of the intracellular background of these compounds /15, 16/, and also the fact that not all exogenous substances can penetrate through the cell membranes.

Differences in the incorporation of AMP and ATP in RNA and DNA are apparently explained by their different capacity to convert into droxyribonucleotides.

Conclusions

1. The plague microbe (EV strain), in spite of the capacity to synthesize purines de novo and the presence in the cells of a background of precursors of purines of nucleic acids, readily incorporates exogenous purines into the polynucleotides; these are adenine, guanine, hypoxanthine, and xanthine. All or almost all the adenine of freshly synthesized nucleic acids is formed from exogenous adenine. Exogenous xanthine may be the main source of guanine of nucleic acids.

2. The plague microbe does not possess the capacity to convert exogenous adenine to guanine and weakly converts exogenous guanine to adenine of polynucleotides.

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